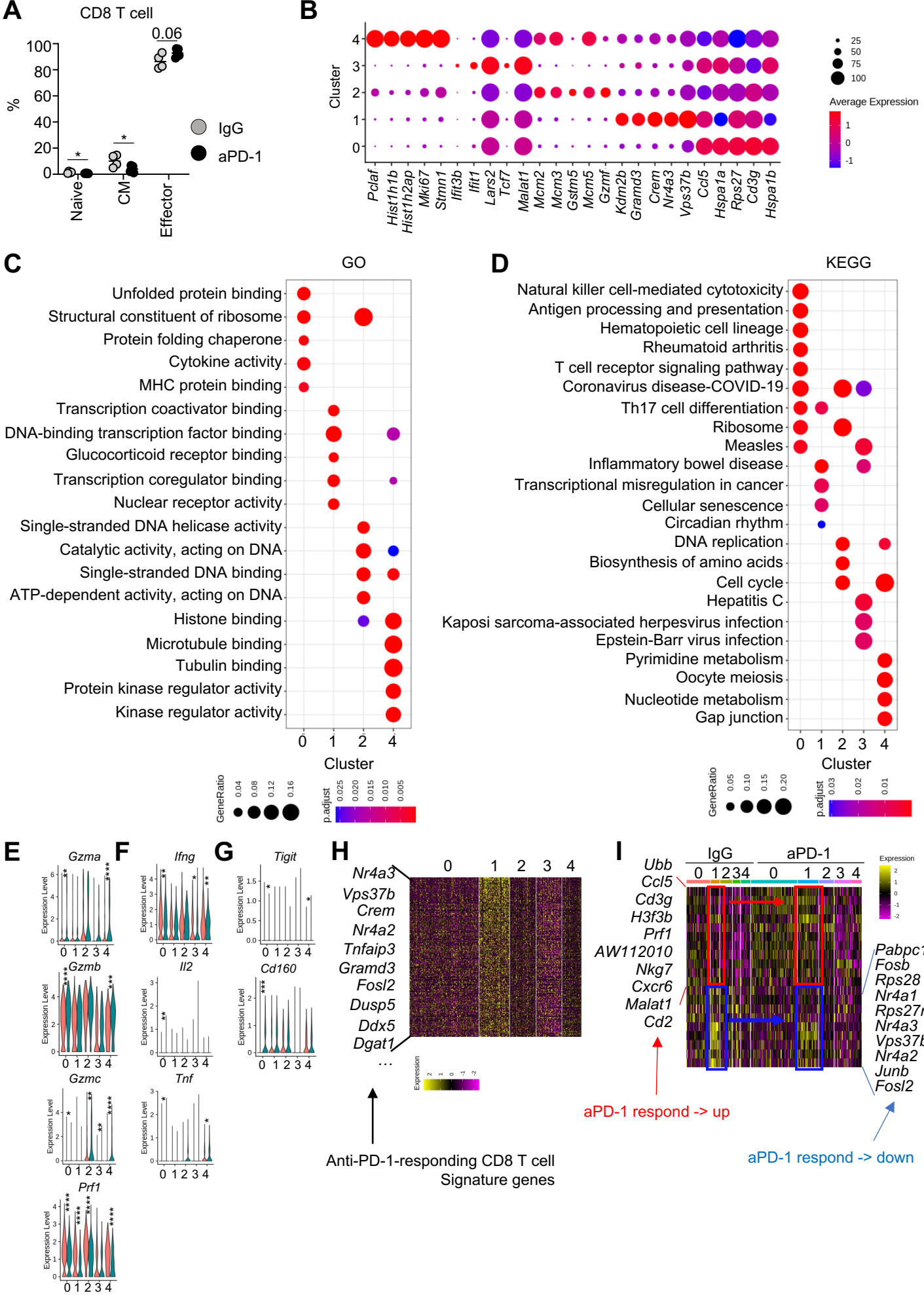


Figure S1. Immune reaction to aPD-1 treatment in GL261-injected mice, related to Figure 1.

(A) Experimental scheme. Eight-week-old male C57BL/6J mice were intracranially injected with GL261 cells. At 11, 13, and 15 days post tumor injection, 200 μ g aPD-1 or IgG control was intraperitoneally injected into the mice. At 20 days post tumor injection, the mice were sacrificed and analyzed. (B) Gating strategy for immune cell sorting from tumor tissues. (C) Expression of marker genes for identification of clusters. (D) UMAPs of cells from IgG- and aPD-1-treated mice were overlapped. (E) Frequencies of clusters from IgG- or aPD-1-treated groups were compared. (F–H) At 20 days post tumor injection, IgG- or aPD-1-treated mice were sacrificed, and cells were isolated and analyzed by flow cytometry. (F) Gating strategy. (G) The numbers of CD45⁺ cells, tumor-infiltrating leukocytes (TILs; CD45^{hi}), CD4 T cells (CD45^{hi}CD3e⁺CD4⁺), and CD8 T cells (CD45^{hi}CD3e⁺CD8a⁺) were analyzed. (H) MuLV tetramer-specific CD8 T cells were analyzed. Gating (upper) and the number of tetramer-specific CD8 T cells (below) are shown. Data in G and H were analyzed by unpaired, two-tailed Student's *t* test. **P*<0.05, ***P*<0.01. Error bars represent the mean \pm standard error of the mean (SEM).

Figure S2. PD-1 expression in immune cells from GBM tissues, related to Figure 2.

(A) Gating strategy for Figure 2. (B) Expression density of *ITGAM*, *CD3E*, *CD4*, *CD8A*, *FOXP3*, *MALAT1*, *GFAP*, and *SOX2* from scRNAseq data GSE154795. (C) Overall survival of patients with GBM from the CGGA database divided by *PDCDI* expression (divided by median value of 0.33; 69 patients for the low group and 67 patients for the high group). (D) Overall survival of patients with GBM from the GEPIA database divided by *PDCDI* expression (divided by median value; 80 patients for the high group and 78 patients for the low group). Survival data were analyzed by log-rank test. * $P < 0.05$, ** $P < 0.01$



Park and Kang, Figure S3

Figure S3. The landscape of CD8 T cells in brain tumors, related to Figure 3.

(A) Eight-week-old male C57BL/6J mice were intracranially injected with GL261 cells. After three treatments with aPD-1, at 20 days post tumor injection, cells were isolated and analyzed by flow cytometry. The percentages of naïve (CD44⁺CD62L⁺), central memory (CM; CD44⁺CD62L⁺), and effector (CD44⁺CD62L⁻) cells among the CD8 T cells were analyzed. (B–I) CD8 T cells (*Cd3e*⁺*Cd8a*⁺*Cd4*-doublet⁻) were isolated from scRNAseq data and analyzed. (B) Dot plot for marker genes of CD8 T-cell clusters. (C, D) DEGs of clusters were analyzed to find enriched pathways using the GO (C) and KEGG (D) databases. (E–G) The expression of *Gzma*, *Gzmb*, *Gzmc*, *Prfl* (E), *Ifng*, *Il2*, *Tnf* (F), *Tigit*, and *Cd160* (G). (H) DEGs from cluster 1 compared with others were designated as “anti-PD-1-responding CD8 T-cell signature genes” and visualized by heatmap. (I) DEGs of cluster 1 from the aPD-1 group (red) and the IgG group (blue) were visualized by heatmap. Data in A were analyzed by unpaired, two-tailed Student’s *t* test. Data in E–G were analyzed by the `stat_compare_means` function with unpaired t-test. **P*<0.05. Error bars represent the mean ± standard error of the mean (SEM).

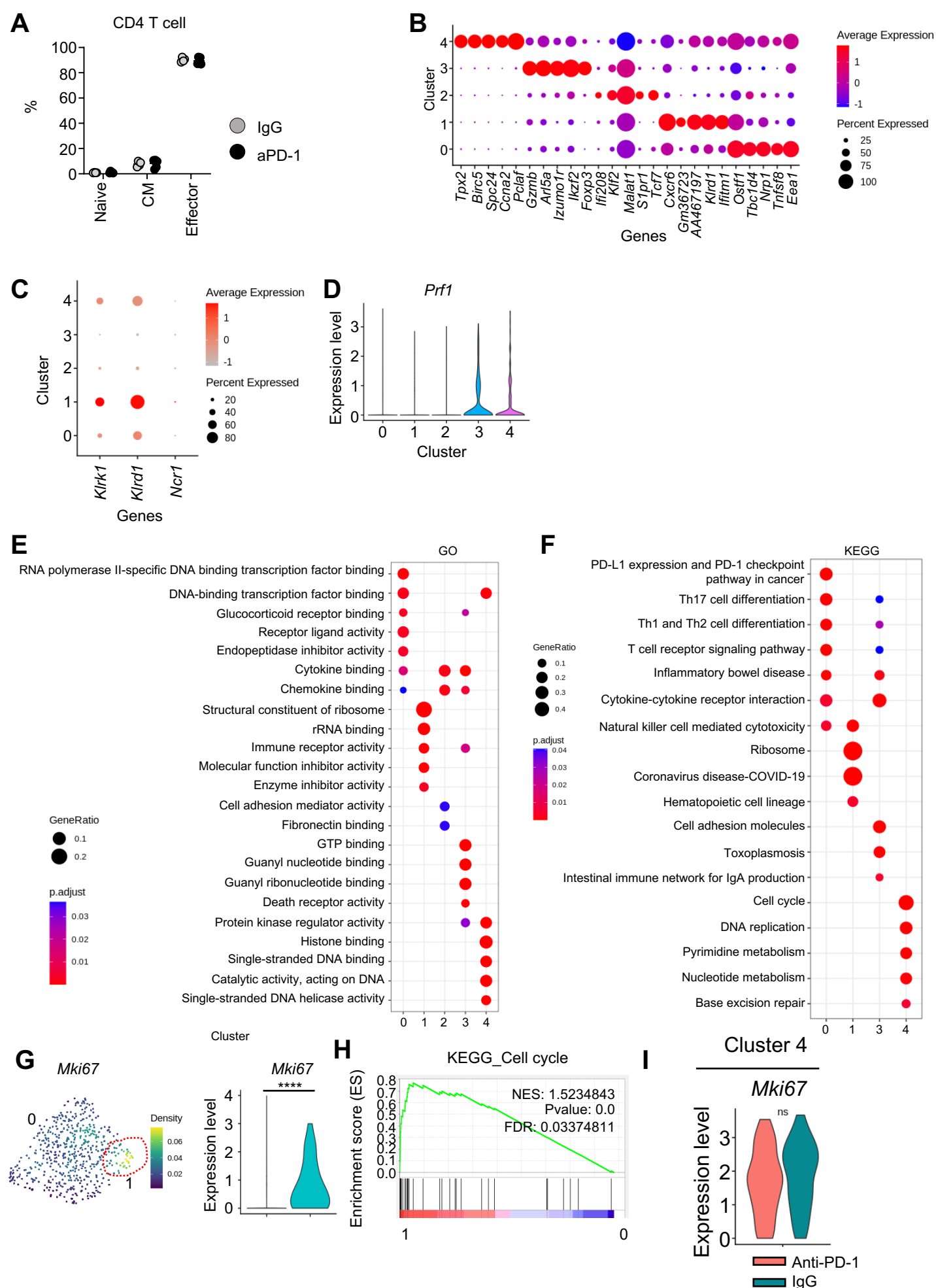
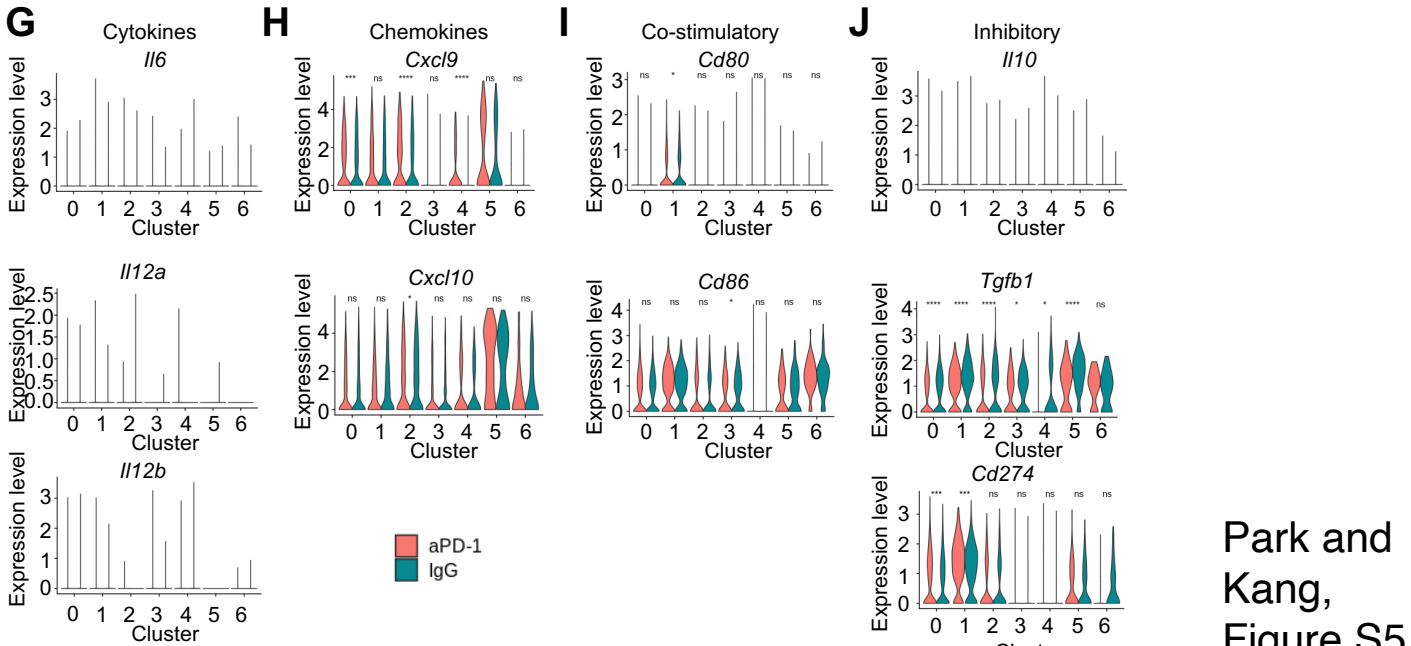
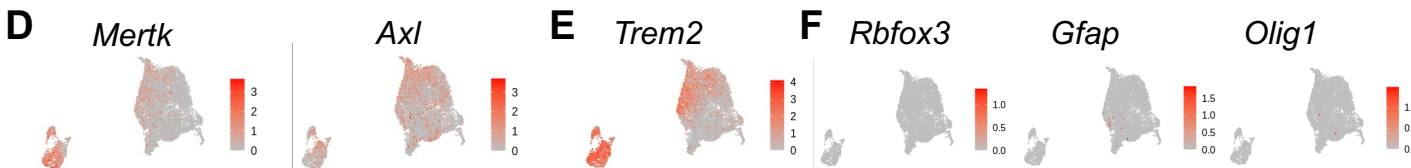
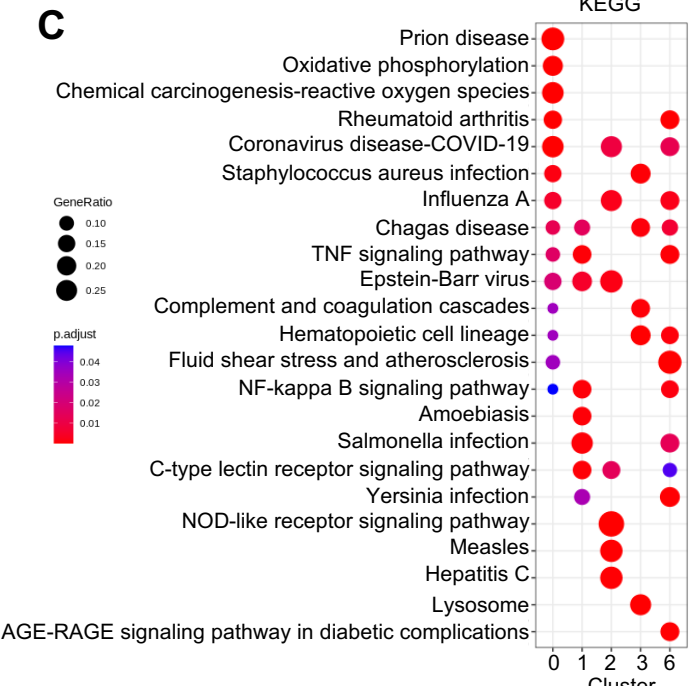
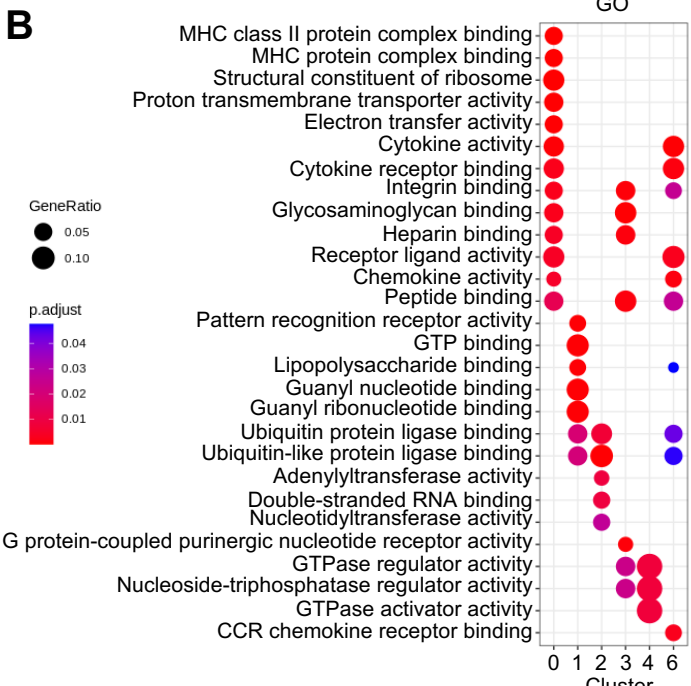
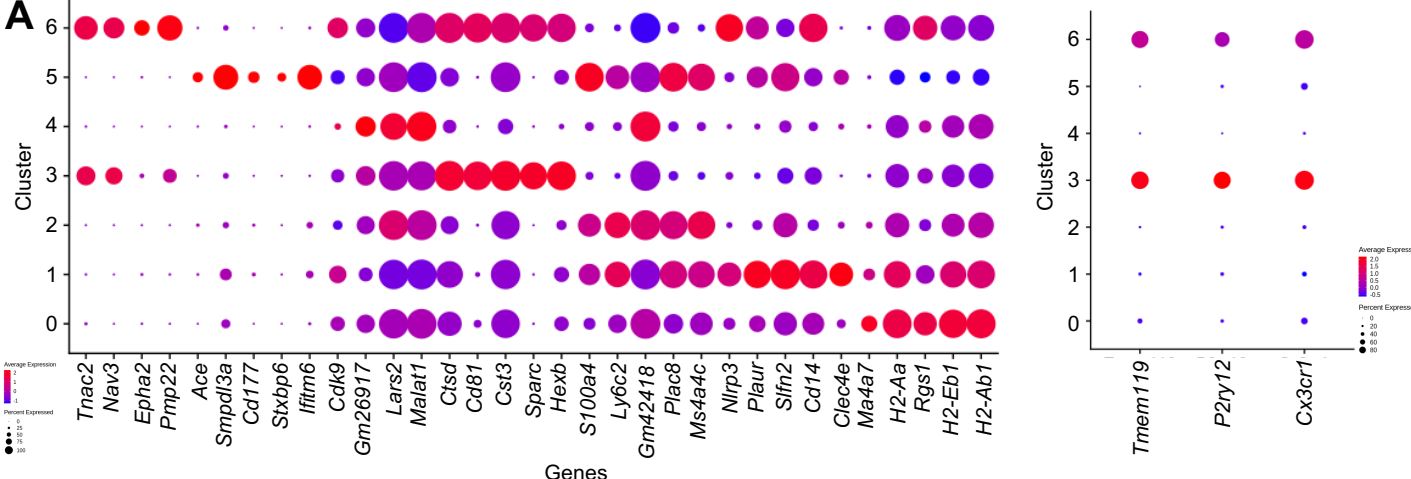


Figure S4. The CD4 T-cell transcriptome in brain tumors, related to Figure 4.

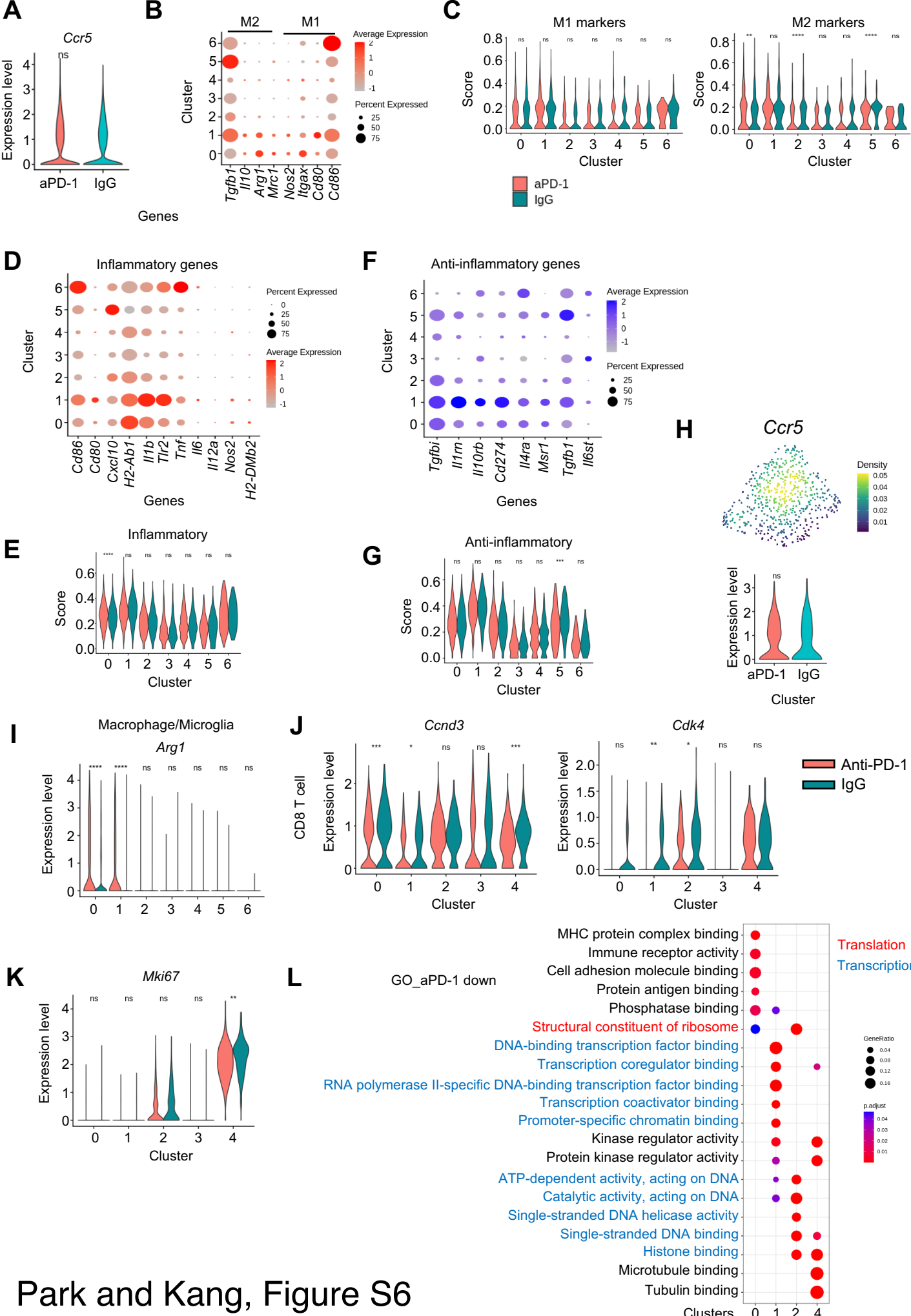
(A) Eight-week-old male C57BL/6J mice were intracranially injected with GL261 cells. After three treatments with aPD-1, cells were isolated and analyzed by flow cytometry at 20 days post tumor injection. The percentages of naïve (CD44⁺CD62L⁺), central memory (CM; CD44⁺CD62L⁺), and effector (CD44⁺CD62L⁻) cells among the CD4 T cells were analyzed. (B–F) CD4 T cells (*Cd3e*⁺*Cd4*⁺*Cd8a*⁻doublet⁻) were isolated from scRNAseq data and analyzed. (B) Marker genes of clusters are displayed by dot plot. (C) Expression of *Klrk1*, *Klrd1*, and *Ncr1* from CD4 T-cell clusters was analyzed. (D) *Prfl* expression from clusters was analyzed. (E, F) Enriched pathways from CD4 T-cell clusters were analyzed based on the GO (E) and KEGG (F) databases. (G, H) Tregs (*Cd3e*⁺*Cd4*⁺*Foxp3*⁺) were isolated from scRNAseq data. (G) *Mki67* expression density and level were analyzed. (H) DEGs from Treg cluster 1 compared to Treg cluster 0 were analyzed using KEGG_Cell cycle gene sets. (I) The *Mki67* expression level of CD4 T cell cluster 4 was analyzed. Data in A were analyzed by unpaired, two-tailed Student's *t* test. Data in G and I were analyzed by the `stat_compare_means` function with unpaired t-test. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Error bars represent the mean ± standard error of the mean (SEM). NES: normalized enrichment score; FDR: false discovery rate.



Park and Kang, Figure S5

Figure S5. Transcriptomes of microglia and macrophages in the brain tumor tissue, related to Figure 5.

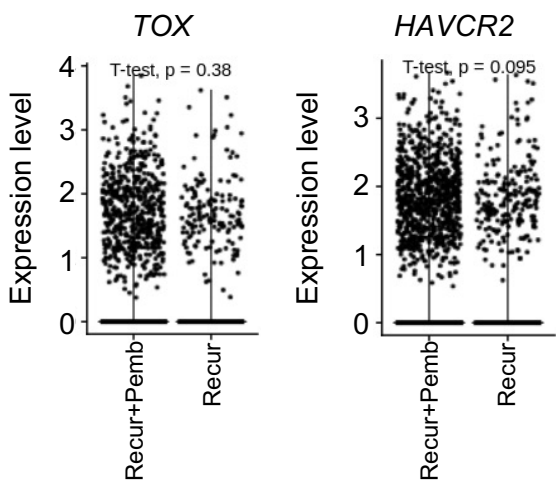
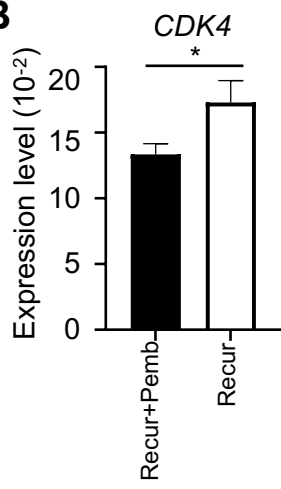
(A) Microglia and macrophage clusters were isolated from scRNAseq data. After re-clustering, marker genes of clusters were analyzed. (B-C) Enriched pathways based on DEGs of clusters were analyzed using the GO (B) and KEGG (C) databases. (D-F) Expression of *Mertk* and *Axl* (D), *Trem2* (E), and *Rbfox3*, *Gfap*, and *Olig1* (F) is shown by feature plots. (G-J) Expression of cytokines (*Il6*, *Il12a*, *Il12b*) (G), chemokines (*Cxcl9*, *Cxcl10*) (H), co-stimulatory genes (*Cd80*, *Cd86*) (I), and inhibitory genes (*Il10*, *Tgfb1*, *Cd274*) (J) from macrophage/microglia clusters was analyzed. Data were analyzed by the `stat_compare_means` function with unpaired t-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.



Park and Kang, Figure S6

Figure S6. Immunosuppressive effects of aPD-1 treatment, related to Figure 6.

(A) Expression of *Ccr5* in total cells from scRNAseq data was analyzed. (B, C) Expression level (B) and score (C) of M1 and M2 markers from macrophage/microglia subclusters. (D–E) Expression level (D, F) and score (E, G) of inflammatory (D, E) and anti-inflammatory (F, G) genes in macrophage/microglia subclusters. (H) *Ccr5* expression density and level in Treg subclusters were analyzed. (I) Expression of *Arg1* in macrophage/microglia subclusters. (J–K) Expression of *Ccnd3* and *Cdk4* (J) and *Mki67* (K) in CD8 T-cell subclusters was analyzed. (L) Pathways enriched in CD8 T-cell subclusters from IgG-treated mice compared with aPD-1-treated mice were analyzed using the GO database. Translation-related pathway (red), transcription-related pathways (blue), and other pathways (black). Data were analyzed by the `stat_compare_means` function with t-test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

A**B****C**

CGGA WHO IV primary GBM

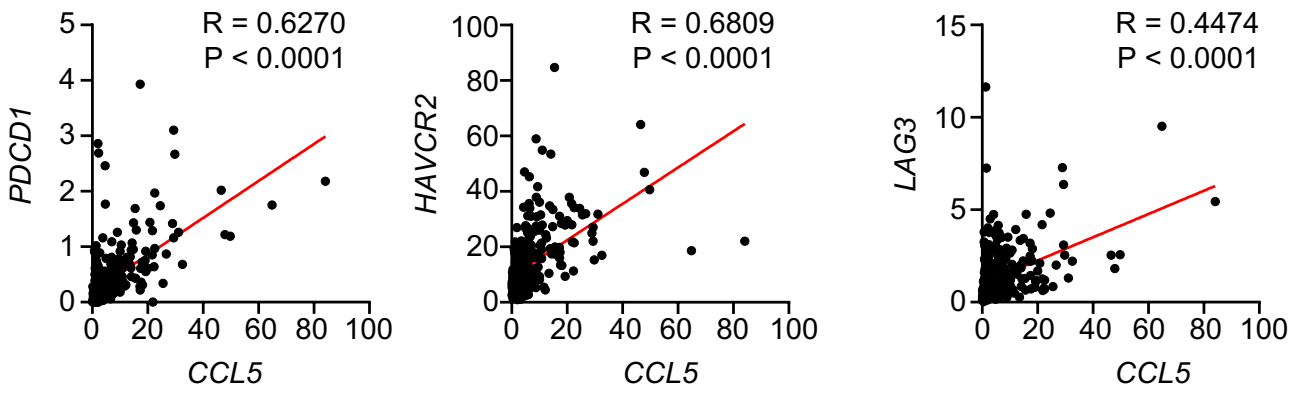
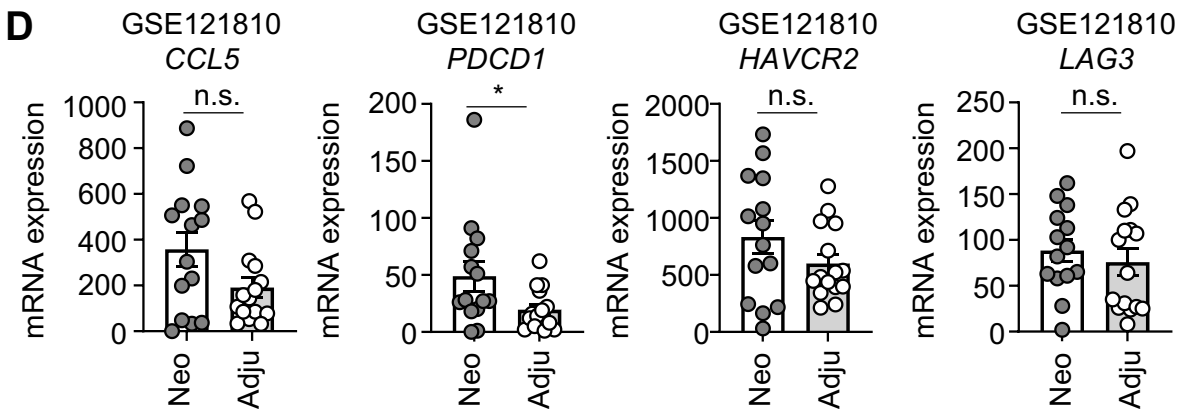
**D**

Figure S7. The effect of aPD-1 in human patients, related to Figure 7.

(A) Expression of *TOX* and *HAVCR2* in CD8 T-cell clusters of the GSE154795 data set. (B) Expression of *CDK4* in CD8 T-cell clusters of the GSE154795 dataset. (C) Using WHO grade IV primary GBM patient data from the CGGA database, correlations between *CCL5* and *PDCD1*, *HAVCR2*, and *LAG3* were analyzed. (D) Using RNAseq GSE121810 data, expression of *CCL5*, *PDCD1*, *HAVCR2*, and *LAG3* was compared between neoadjuvant and adjuvant pembrolizumab-treated samples. Data in A were analyzed by the `stat_compare_means` function with t-test. Data in B and D were analyzed by unpaired, two-tailed Student's *t* test. Data in C were analyzed by Spearman correlation test. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Error bars represent the mean ± standard error of the mean (SEM).

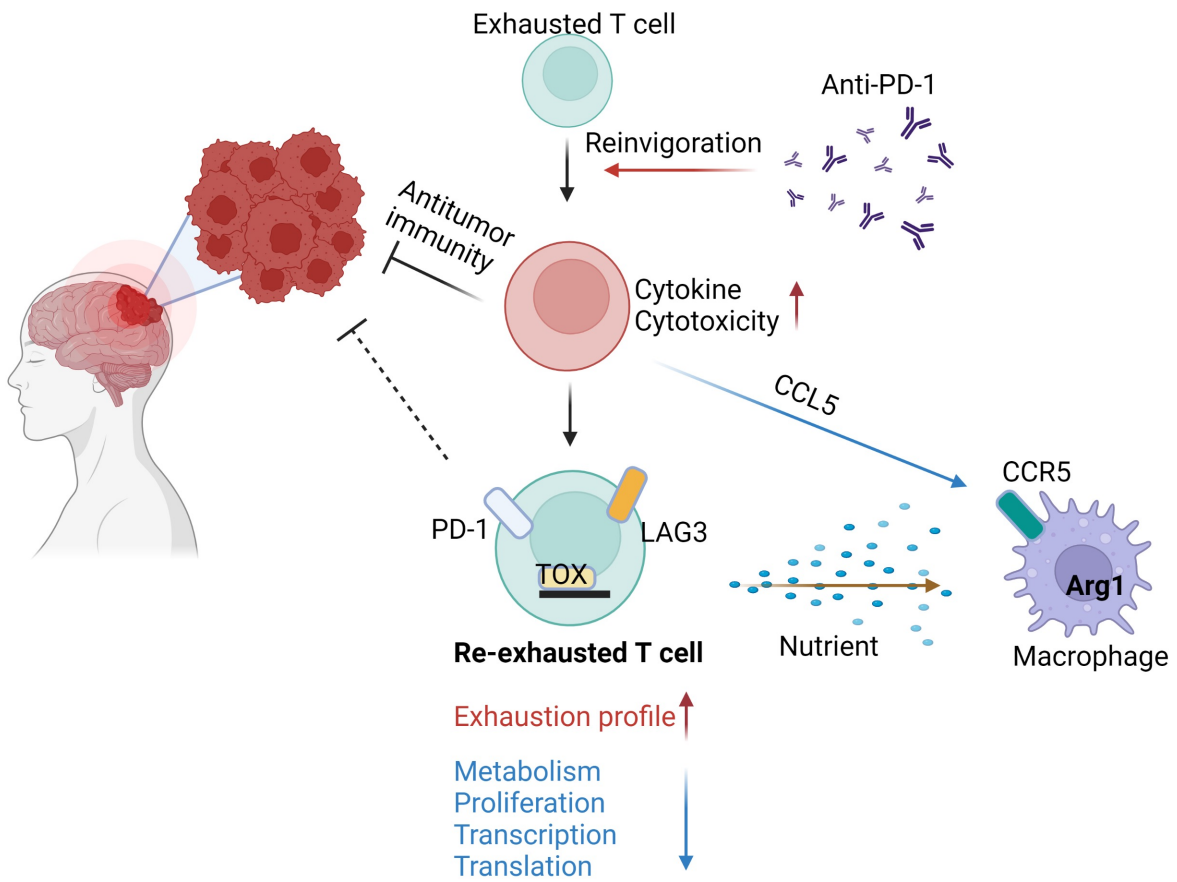


Figure S8. Scheme of mechanisms.

Within the brain tumor microenvironment, T cells are exhausted. If anti-PD-1 therapy is given, CD8 T cells can be reinvigorated by upregulation of cytokines and cytotoxicity molecules. Reinvigorated T cells also express CCL5, which recruits macrophages. CCR5⁺ macrophages are anti-inflammatory and hijack nutrients from T cells via Arg1. As a result, T cells are re-exhausted. Re-exhausted T cells are marked by expression of PD-1, LAG3, and TOX. These cells show increased exhaustion profiles as pathways related to metabolism, proliferation, transcription, and translation are downregulated. This image was created with BioRender.com